Mass spectrometry and ribosome profiling, a perfect combination towards a more comprehensive identification strategy of true *in vivo* protein forms.

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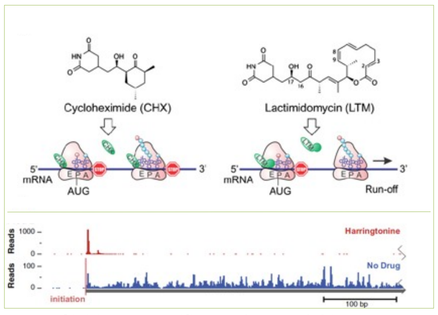
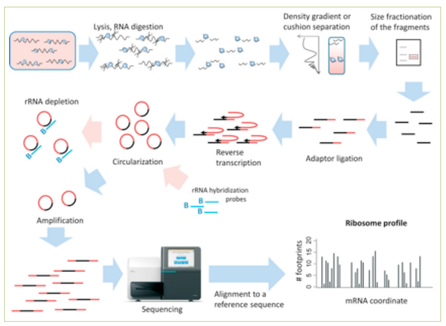
INTRODUCTION

An increasing number of studies involve **integrative analysis of gene and protein expression data**, taking advantage of new technologies such as next-generation transcriptome sequencing (**RNA-Seq**) and highly sensitive mass spectrometry (**MS**). Recently, a strategy, termed **ribosome profiling**, based on deep sequencing of ribosome-protected mRNA fragments, indirectly **monitoring protein synthesis**, has been described. When used in combination with **initiation-specific translation inhibitors**, it enables the identification of (alternative) translation initiations.



MOA of initiation-specific translation inhibitors and example of gene-mapped RIBO-seq signals [1,2]



RIBO-seq experimental workflow [3]

In contrast to routinely employed protein databases in proteomics searches, RIBO-seq derived data gives a **more representative expression state** and accounts for sequence variation information (**single nucleotide polymorphism, insertions, deletions and RNA-splice variants**) and **alternative translation initiation** leading to N-terminal **extended and/or truncated protein forms**. Furthermore, RIBO-seq reveals translation start at **near-cognate start sites**. Without taking this information into account, MS-based proteomic studies may fail to detect novel, important protein forms.

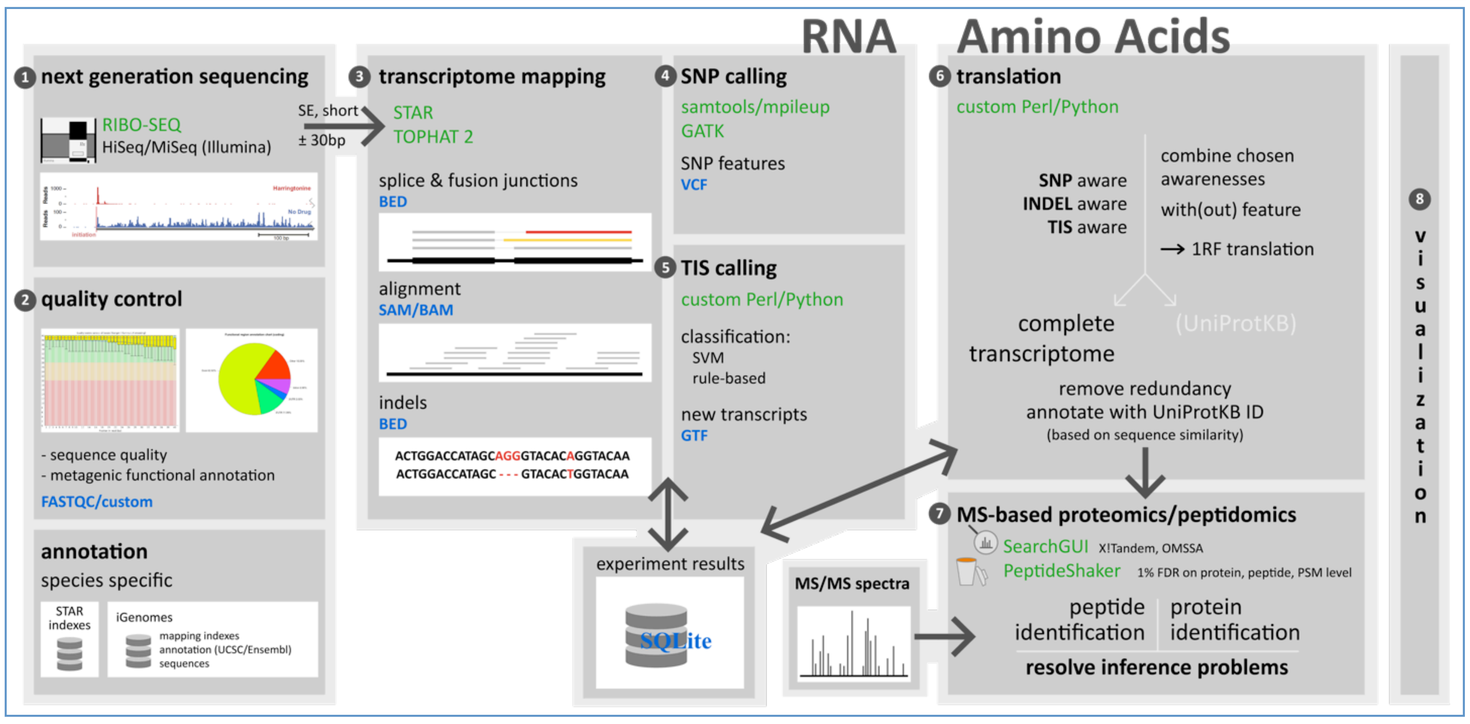
GOALS

✓ Compile a **sample-specific protein search database** based on ribosome profiling sequencing data.

✓ Introduce **new translation products** in the MS search space: N-terminal extensions/ truncations, trans-lated uORFs, near-cognate start sites.

✓ Bridging two omics worlds: **transcriptomics** & MS-based **proteomics** by means of **RIBO-seq**.

MATERIALS & METHODS



WORKFLOW OVERVIEW

1. Ribosome profiling of ribosome captured mRNA fragments performed on HiSeq, Illumina sequencing platform (**RIBO-seq**).

2. **Quality control** based on existing tools as FastQC and custom metagenic functional assessment.

3. **Transcriptome mapping** based on STAR [4] and/or TopHat2 [5] local aligners.

4. Single nucleotide polymorphism **(SNP) calling** based on samTools-mpileup and/or GATK.

5. Translation initiation site **(TIS) calling** based on trained Support Vector Machine (SVM) [2] or rule-based algorithm [1].

6. **Translation product assembly**, taken into account the SNP, TIS, INDEL awareness. Construct complete proteome, optionally combine with SwissProt.

7. **MS-based proteomics/peptidomics** using SearchGui [6] and PeptideShaker [7] tools.

8. **Genome-centric visualization** of all generated information tracks (Ensembl, UCSC, IGV genome browsers).

=> All results are stored in a ***relational SQLite database*** allowing further detailed analysis.

RESULTS

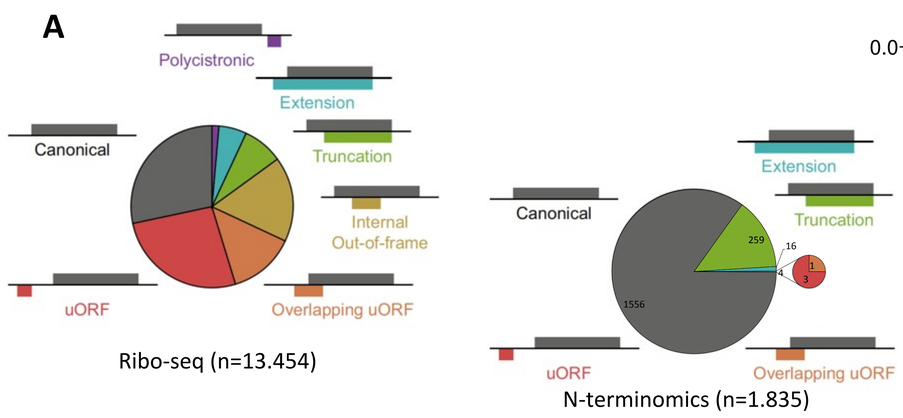
MS experiments - 2 Data Sets:

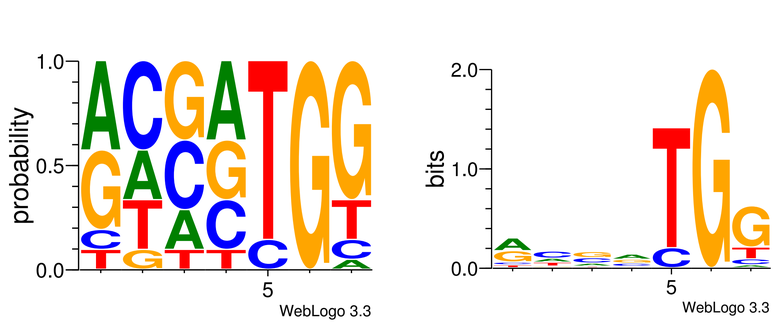
Shotgun proteomics: 24 LC runs -> 112.974 MS/MS spectra.

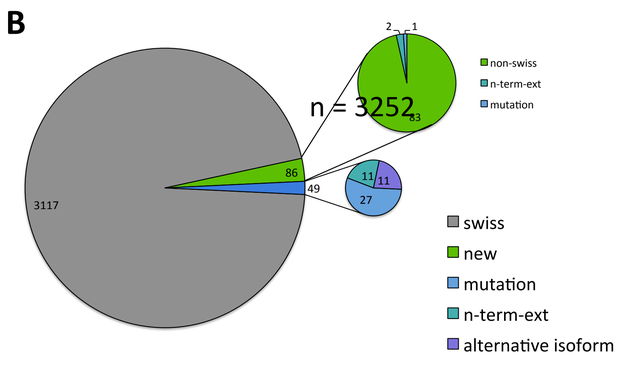
Positional proteomics (N-term COFRADIC): 45 LC runs -> 68.523 MS/MS spectra

Version 1 Custom DB [8] based on:

- **SVM based TIS calling** [2]
- **Using reference genomic sequence**
- **Combi of RIBO-seq derived and SwissProt**



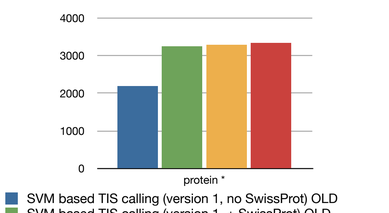




(**A**) COFRADIC: Pie charts depicting the **identification of novel translation products**: N-terminal extensions truncations, uORF translations, internal out-of-frame translations (left panel is RIBO-seq based, right panel is MS-based using custom DB). (**B**) SHOTGUN: Pie chart showing an +2.5% **gain in protein identification** using the combined RIBO-seq derived and SwissProt search database. (**C**) Weblogos depicting the sequence context (three bases upstream and four bases downstream) of the newly identified translation initiation sites, clearly pointing to **near-cognate translation initiation**.

Version 2 Custom DB based on:

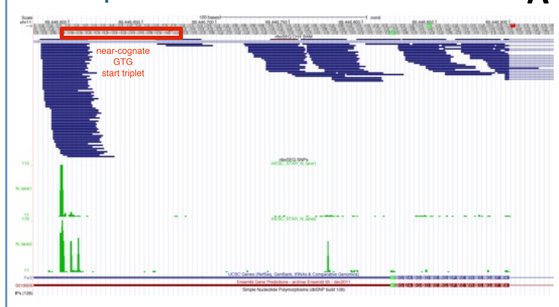
- **rule-based TIS calling** [1]
- **Including SNP-INDEL information**
- **Only Ribo-Seq derived sequences**

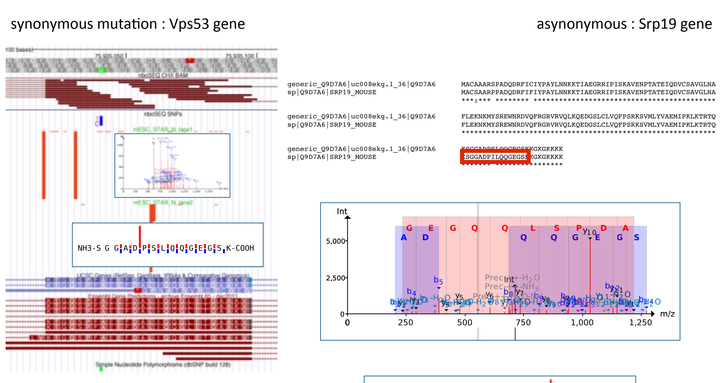


shotgun identifications using custom protein DB	protein *	peptide *	PSM *
SVM based TIS calling (version 1, no SwissProt) OLD	2194	14519	32818
SVM based TIS calling (version 1, + SwissProt) OLD	3252		
stringent rule based TIS calling NEW	3295	15913	32348
stringent rule based TIS calling and SNP/INDEL aware NEW	3341	14967	32620

** 1% FDR level on protein, peptide and PSM level*

Examples:





(**A**) UCSC genome-browser screenshot showing the **extended form of the Fxr2 gene** translation product starting at **near-cognate GTG start site**. Identified tryptic peptide is also depicted. (**B**) 2 examples of (a)syn-onymous **SNPs** with overlapping tryptic peptide identification resulting from the custom RIBO-seq derived protein product database.

CONCLUSION & FUTURE WORK

✓ Deep proteome coverage based on **ribosome profiling aids mass spectrometry-based protein and peptide discovery** and provides evidence of **alternative translation products** and **near-cognate translation initiation events** [1].

✓ Future work will mainly focus on :

➔Further investigation of the differential expression on translation level of UniProtKB-SwissProt and RIBO-seq derived translation products: technological and/or biological relevance? Detailed assessment of the difference between *in vivo* measurement of **protein synthesis** (RIBO-seq) and **protein presence** (MS-based proteomics).

➔**Generalize the pipeline** to all types of next-generation sequencing RNA-seq data: (directional) A+/A- RNA-seq, CLIP-seq, exome-seq, ribo-seq

➔**Quantitative correlation** of RIBO-seq and (non) labelled MS-based proteomics

➔Incorporate Pipeline into **Galaxy-P** [9]

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